

## Claims

5 1. A transgenic *Drosophila* whose genome comprises the full-length human colon cancer gene *Adenomatous Polyposis Coli* (APC) having SEQ ID NO.1 wherein:

(a) said genomic alteration allows mis-expression of full-length human APC in flies in regulated manner,

10 (b) said mis-expression of the full-length human APC results in developmental abnormalities,

(c) said developmental abnormalities induced by the mis-expression of full-length human APC in flies are similar to those exhibited by flies carrying mutations in *Drosophila wingless* gene, and

15 (d) to use the same as an assay system for screening and validating efficacy of drugs.

2. The transgenic *Drosophila* as claimed in claim 1 wherein, its genome includes  $\beta$ -catenin binding domain comprising of amino-acids from 959 to 1870 of SEQ ID NO. 2 from the full length human APC gene of SEQ ID NO.1, and this engineered disruption of human APC comprises only the five of the seven  $\beta$ -catenin binding domains wherein:

(a) said genomic alteration allows mis-expression of a truncated version of human APC in flies in a regulated manner,

25 (b) said mis-expression of the said gene construct results in the developmental abnormalities,

(c) said developmental abnormalities induced by the mis-expression of the said gene construct in flies is similar to those exhibited by flies carrying mutations in *Drosophila wingless* gene,

30 (d) said mis-expression of the said novel construct in regulated manner results in a more severe developmental phenotype, and

(e) to use the same as an assay system for screening and validating efficacy of drugs.

3. The transgenic *Drosophila* as claimed in claim 1 wherein, the N terminal domain of APC with amino acids from 1 to 767 having SEQ ID NO. 3, from the full length human APC gene of SEQ ID NO.1, wherein:

(a) said genomic alteration allows mis-expression of human APC in flies in a regulated manner,

(b) said mis-expression of the said novel construct in a regulated manner resulting in severe abnormalities in fly development during metamorphosis, and

(c) to use the same as an assay system for screening and validating efficacy of drugs.

4. A method for selecting a compound for pharmacological activity, which potentially inhibits or enhances the developmental abnormalities induced by the expression of full length and protein domains of human APC in *Drosophila*, said method comprising:

(a) providing the first, second, and third transgenic fly of claims 1, 2 and 3 respectively, wherein said flies have said developmental abnormalities,

(b) administering the said compounds to the said transgenic *Drosophila* at different concentrations, and

(c) screening for the changes in the severity of the phenotype .

5. A method of determining various *Drosophila* proteins interacting with full-length and protein domains human APC protein wherein, said method comprising:

- (a) providing the first, second, and third transgenic fly of claims 1, 2 and 3 respectively, wherein said flies have said developmental abnormalities,
- (b) crossing the said transgenic flies individually to a set of *Drosophila* strains each of which carries mutation in a different gene or set of genes, and
- (c) Screening for the change in the severity of the phenotype.

5

6. A method for determining the modulation and differential expression of genes following the mis-expression of full-length and its protein domains human APC in *Drosophila* wherein, said method comprising:

10

- (a) providing the transgenic *Drosophila* as claimed in claims 1,2 and 3 wherein, the flies have developmental abnormalities,
- (b) screening for differential gene expression using differential display-RT PCR or microarray techniques, and
- 15 (c) identifying genes that are differentially regulated on expression of human APC.

7. A method for determining the modulation and differential expression of proteins following the mis-expression of full-length and its protein domain human APC in *Drosophila* wherein, said method comprising:

20

- (a) providing the transgenic *Drosophila* , as claimed in claims 1, 2 and 3 wherein, the flies have developmental abnormalities,
- (b) identifying differential gene expression and protein modifications using proteomics techniques, and
- 25 (c) identifying gene products that are differentially regulated on expression of human APC.

8. A method to study Wnt/Wg signaling in *Drosophila* said method comprising;
- (a) providing the transgenic *Drosophila*, as claimed in claims 1, 2 and 3,
  - (b) crossing these transgenic flies to a number of GAL4 drivers to induce targeted expression of said constructs in various tissues and at different developmental stages, and
  - (c) examining developmental abnormalities.
9. Methods as claimed in claims 6–8 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to study mechanism of various developmental processes such as wing, leg, eye, antennae, and adult cuticle development.
10. A Method as claimed in claim 4 wherein, screening and validating efficacy of preventive and therapeutic drugs following APC gene mis-expression.
11. A Method as claimed in claim 4 wherein, human APC pathway is identified using drug selected from a group of compounds comprising anti inflammatory, Analgesics, Antipyretics, and Antineoplastics.
12. A method as claimed in claim 4 wherein, concentration of said drugs ranging between 50 to 500 µg/ml of fly food.
13. Methods as claimed claims 6-8 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC which has advantages to study the *Drosophila* Wnt/Wg signaling pathway.
14. A Method as claimed in claim 8 wherein, studying the kinetics of Wnt/Wg signaling during various developmental stages and in different tissues.

15. A Method as claimed in claims 5 and 7 wherein, new target proteins interacting with  $\beta$ -catenin are identified.

16. A Method as claimed in claim 6 wherein, genes interacting with APC are identified.

17. Methods as claimed in claims 5–8 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to study biochemical function of human APC function.

18. Methods as claimed in claims 5–8 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to identify additional components of *Drosophila* Wnt/Wg signaling pathway.

19. A transgenic *Drosophila* whose genome comprises the full-length human colon cancer gene *Adenomatous Polyposis Coli* (APC) having SEQ ID NO.1 wherein:

(a) said genomic alteration allows mis-expression of full-length human APC in flies in regulated manner,

(b) said mis-expression of the full-length human APC results in developmental abnormalities,

(c) said developmental abnormalities induced by the mis-expression of full-length human APC in flies are similar to those exhibited by flies carrying mutations in *Drosophila wingless* gene, and

(d) to use the same as an assay system for screening and validating efficacy of anti-cancer drugs.

20. The transgenic *Drosophila* as claimed in claim 19 wherein, its genome includes  $\beta$ -catenin binding domain comprising of amino-acids from 959 to 1870 of SEQ ID NO. 2 from the full length human APC gene of SEQ ID NO.1, and this engineered disruption of human APC comprises only the five of the seven  $\beta$ -catenin binding domains wherein:

- (a) said genomic alteration allows mis-expression of a truncated version of human APC in flies in a regulated manner,
- (b) the mis-expression of the said gene construct results in the developmental abnormalities,
- 5 (c) the developmental abnormalities induced by the mis-expression of the said gene construct in flies is similar to those exhibited by flies carrying mutations in *Drosophila wingless* gene,
- (d) mis-expression of the said novel construct in regulated manner results in a more severe developmental phenotype, and
- 10 (e) to use the same as an assay system for screening and validating efficacy of anti-cancer drugs.

21. The transgenic *Drosophila* as claimed in claim 19 wherein, the N terminal domain of APC with amino acids from 1 to 767 having SEQ ID NO. 3, from the full length human APC gene of SEQ ID NO.1 wherein:

15

- (a) the said genomic alteration allows mis-expression of human APC in flies in a regulated manner,
- (b) the mis-expression of the said novel construct in a regulated manner resulting in severe abnormalities in fly development during metamorphosis, and
- 20 (c) to use the same as an assay system for screening and validating efficacy of anti-cancer drugs.

22. A method for selecting a compound for anti-cancer activity, which potentially inhibits or enhances the developmental abnormalities induced by the expression of full length and protein domains of human APC in *Drosophila*, said method comprising:

25

- (a) providing the first, second, and third transgenic fly of claims 19, 20, and 21 respectively, wherein said flies have said developmental abnormalities,
- 30

- (b) administering the said compounds to the said transgenic *Drosophila* at different concentrations, and
- (c) screening for the change in the severity of the phenotype .

5 23. A method of determining various *Drosophila* proteins interacting with full-length and protein domains human APC protein wherein, said method comprising:

- 10 (a) providing the first, second, and third transgenic fly of claims 19, 20, and 21 respectively, wherein said flies have said developmental abnormalities,
- (b) crossing the said transgenic flies individually to a set of *Drosophila* strains each of which carries mutation in a different gene or set of genes, and
- 15 (c) Screening for the change in the severity of the phenotype.

24. A method for determining the modulation and differential expression of genes following the mis-expression of full-length and its protein domains human APC in *Drosophila* wherein, said method comprising:

- 20 (a) providing the transgenic *Drosophila* as claimed in claims 19,20, and 21 wherein, the flies have developmental abnormalities,
- (b) screening for differential gene expression using differential display-RT PCR or microarray techniques, and
- 25 (c) identifying genes that are differentially regulated on expression of human APC.

25. A method for determining the modulation and differential expression of proteins following the mis-expression of full-length and its protein domain human APC in *Drosophila* wherein, said method comprising:

- (a) providing the transgenic *Drosophila* , as claimed in claims 19, 20, and 21 wherein, the flies have developmental abnormalities,
- (b) identifying differential gene expression and protein modifications using proteomics techniques, and
- 5 (c) identifying gene products that are differentially regulated on expression of human APC.

26. A method to study Wnt/Wg signaling in *Drosophila* said method comprising;

- (a) providing the transgenic *Drosophila*, as claimed in claims 19-21,
- 10 (b) crossing these transgenic flies to a number of GAL4 drivers to induce targeted expression of said constructs in various tissues and at different developmental stages, and
- (c) examining developmental abnormalities.

15 27. Methods as claimed in claims 24-26 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to study mechanism of various developmental processes such wing, leg, eye, antennae, and adult cuticle development.

20 28. A Method as claimed in claim 22 wherein, screening and validating efficacy of anti-cancer drugs following APC gene mis-expression.

25 29. A Method as claimed in claim 22 wherein, human APC pathway is identified using drugs selected from a group of compounds comprising anti inflammatory, Analgesics, Antipyretics, and Antineoplastics.

30 30. A method as claimed in claim 22 wherein, concentration of said anti-cancer drugs ranging between 50 to 500 µg/ml of fly food.



31. Methods as claimed claims 24-26 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC which has advantages to study the *Drosophila* Wnt/Wg signaling pathway.
- 5 32. A Method as claimed in claim 26 wherein, studying the kinetics of Wnt/Wg signaling during various developmental stages and in different tissues.
33. Methods as claimed in claims 23 and 25 wherein, new target proteins interacting with  $\beta$ -catenin are identified.
- 10 34. A Method as claimed in claim 24 wherein, genes interacting with APC are identified.
35. Methods as claimed in claims 23-26 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to study biochemical function of human APC function.
- 15 36. Methods as claimed in claims 23-26 wherein, examination of developmental abnormalities using gain-of-function genetic model for human APC to identify additional components of *Drosophila* Wnt/Wg signaling pathway.
- 20